

CLAIMS

1. A method of introducing genes into cotton plants and plant lines comprising exposing hypocotyl tissue of immature cotton plants to a culture of transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene.

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2. The method of Claim 1 further comprising:

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 - culturing the exposed tissue in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed with the T-DNA region;
 - inducing somatic embryo formation in the exposed tissue in culture; and
 - 15 regenerating the somatic embryos into whole cotton plants.
3. The method of Claim 1 wherein said exposing step is preceded by surface sterilization of cotton seeds followed by germination of said cotton seeds to form said immature cotton plants.

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4. The method of Claim 1 wherein the hypocotyl tissue comprises pieces of hypocotyl explants which are removed from said immature cotton plants.
5. The method of Claim 1 wherein the culture of Agrobacterium tumefaciens harbors a binary Ti plasmid system in which a virulence trait is carried on a plasmid separate from the plasmid carrying the T-DNA region.

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6. The method of Claim 5 wherein the T-DNA region includes only the T-DNA right and left borders from the T-DNA of a wild-type Ti plasmid.

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7. The method of Claim 2 wherein the selection agent is an antibiotic and the resistance gene codes for antibiotic resistance.
8. The method of Claim 7 wherein the antibiotic resistance gene is the NPTII gene and the antibiotic is selected from the group consisting of Kanamycin and G418.
9. The method of Claim 7 wherein the antibiotic resistance gene is the CAT gene and the antibiotic is Chloramphenicol.
10. 10. The method of Claim 7 wherein two antibiotics and two antibiotic resistance genes are used.
11. The method of Claim 10 wherein the two antibiotics are selected from the group consisting of Hygromycin B, Chloramphenicol, and one of Kanamycin and G418.
12. The method of Claim 1 further including, after the step of exposing the tissue to said Agrobacterium tumefaciens, culturing the tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells.
13. The method of Claim 2 wherein the step of inducing embryo formation includes culturing the plant tissue on a culture media containing at least one auxin or cytokinin.
14. Cotton plants produced by the method of Claim 2.
15. Cotton somatic embryos produced by the method of Claim 2.

16. Cotton seeds produced by the plants of Claim 14.

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17. Cotton seed capable of germination into cotton plants comprising in their genome a chimeric gene construction including a foreign gene and promoter and control sequences operable in plant cells, the chimeric gene construction being effective in the cells of the cotton plant to express a cellular product coded by the foreign gene.

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10 17. Cotton plants germinated from the seeds of Claim

19. Cotton seeds as claimed in Claim 17 wherein the cellular product is selected from the group consisting of an exogenous protein and an RNA selected to produce a somatic change to the cotton plant.

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15 20. Cotton seeds as claimed in Claim 17 wherein the foreign gene codes for the production of a negative RNA strand effective to condition a somatic change in the cotton plant grown from the seed.

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20 Cotton seeds as claimed in Claim 19 wherein the promoter sequence is selected from the group consisting of the nopaline synthase promoter from Agrobacterium tumefaciens and the cauliflower mosaic virus 35s promoter.

22. A method for introducing genes into cotton plants and plant lines, comprising the following steps in sequence:

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- a) surface sterilizing cotton seeds;
 - b) allowing said cotton seeds to germinate thus forming immature cotton plants, said immature cotton plants including hypocotyl tissue;
 - 30 c) exposing said hypocotyl tissue to a culture of

transformation competent non-oncogenic Agrobacterium tumefaciens harboring a Ti plasmid having a T-DNA region including both a foreign chimeric gene and a selection agent resistance gene;

- 5 d) culturing said hypocotyl tissue on a medium containing at least one antibiotic toxic to said Agrobacterium tumefaciens but not toxic to cotton cells;
- e) culturing said tissue of step d) in the presence of a selection agent for which the resistance gene encodes for resistance so as to select for plant cells transformed
- 10 with the T-DNA region;
- f) inducing somatic embryo formation in the exposed tissue in culture; and
- g) regenerating the somatic embryos into whole cotton
- 15 plants.

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B₂
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